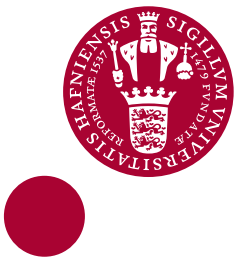

Induction of severe Staphylococcus aureus sepsis in pigs

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of activated fibrinolysis are independently associated with hyperlactatemia. This suggests a contribution of DIC resulting from a coagulation/fibrinolysis imbalance to microvascular obstruction, tissue hypoxygenation and thereby to ultimate demise.

P36

Monitoring of procalcitonin, IL-6 and brain natriuretic peptide for sepsis diagnosis in cardiac surgery

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Introduction Procalcitonin (PCT) and IL-6 are markers used in the evaluation of systemic inflammation (SIRS) and septic states. The purpose of this study is to analyse changes in plasma concentrations of PCT and IL-6 in patients undergoing cardiac surgery on-pump and assess its reliability in the early detection of post-operative infectious complications. In all patients the variation of brain natriuretic peptide (BNP) was also evaluated in order to stratify the clinical condition of patients.
Methods We measured serum levels of PCT, IL-6 and BNP in adult patients undergoing myocardial revascularization and/or valve surgery performed in extracorporeal circulation. The measurements were performed on the day before the intervention (T0), at the end of surgery (T1) and then until the third and fourth postoperative day (T2 to T4). We also recorded the onset of cardiac, respiratory, neurological, renal and septic complications. The diagnosis of sepsis was confirmed retrospectively on the basis of clinical, radiological and microbiological data. All data are expressed as mean and standard deviation. The Kruskal–Wallis test was used to assess changes over time of variables. $P < 0.05$ was considered statistically significant.
Results There have been enrolled 60 patients undergoing cardiac surgery in extracorporeal circulation. Among these, nine patients developed septic complications. The results of temporal changes and the significance are presented in Table 1.

Table 1 (abstract P36)

Results of temporal changes and significance

	T0	T1	T2	T3	T4
PCT (ng/ml)					
Nonseptic	0.04	0.04	0.58	0.34	0.34
Septic	0.04	0.15	2.63	1.87	0.74
P	NS	<0.001	<0.001	<0.001	<0.01
IL-6 (pg/ml)					
Nonseptic	12	160	129	78	75
Septic	18	184	145	261	92
P	NS	NS	NS	<0.01	NS
BNP					
Nonseptic	159	154	347	428	492
Septic	373	627	731	756	798
P	<0.01	< 0.01	<0.01	<0.05	<0.05

Conclusions In patients who develop septic complications, changes in PCT occur earlier than changes in IL-6. Furthermore, BNP performs in the same fashion as PCT and correlates better than IL-6 with the clinical data of the infection status. In conclusion, monitoring PCT seems to be useful in early diagnosis of septic complications in patients undergoing cardiac surgery and more sensitive on the variations in IL-6. The combined study of variations in PCT and BNP could improve the diagnostic accuracy in these patients.

P37

T-cell-specific peroxisome proliferator-activated receptor gamma depletion inhibits T-cell apoptosis and improves survival of septic mice via an IL-2-dependent mechanism

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Introduction Immune paralysis with massive T-cell apoptosis is a central pathogenic event during sepsis and correlates with septic patient mortality. Previous observations implied a crucial role of peroxisome proliferator-activated receptor gamma (PPARγ) during T-cell apoptosis.
Methods To elucidate mechanisms of PPARγ-induced T-cell depletion, we used an endotoxin model as well as the caecal ligation and puncture sepsis model to imitate septic conditions in wild-type versus conditional PPARγ knockout (KO) mice.
Results PPARγ KO mice showed a marked survival advantage compared with control mice. Their T cells were substantially protected against sepsis-induced death and showed a significantly higher expression of the pro-survival factor IL-2. Since PPARγ is described to repress nuclear factor of activated T cells (NFAT) transactivation and concomitant IL-2 expression, we propose inhibition of NFAT as the underlying mechanism allowing T-cell apoptosis. Corroborating our hypothesis, we observed up-regulation of the pro-apoptotic protein BIM and downregulation of the anti-apoptotic protein Bcl-2 in control mice, which are down-stream effector proteins of IL-2 receptor signaling. Application of a neutralizing anti-IL-2 antibody reversed the pro-survival effect of PPARγ-deficient T cells and confirmed IL-2-dependent apoptosis during sepsis.
Conclusions Apparently antagonizing PPARγ in T cells might improve their survival during sepsis, which concomitantly enhances defence mechanisms and possibly provokes an increased survival of septic patients.

P38

Induction of severe *Staphylococcus aureus* sepsis in pigs

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Introduction Organ dysfunction is an integrated part of severe sepsis, and severe sepsis is one of the major causes of death in ICUs. Lately Gram-positive bacteria accounted for more than one-half of the overall sepsis cases reported in the USA, with *Staphylococcus aureus* being the most commonly isolated

bacterium. Effective treatment of sepsis is still not optimal and good animal models are needed for research in pathogenesis and treatment. *S. aureus* infections are also common in pigs and are isolated from approximately 40% of embolic lesions found in slaughter-pigs.

Objective To establish a porcine model of severe sepsis.

Methods Twelve pigs in four groups were inoculated intravenously once or twice with 1 x 10^8 *S. aureus*/kg body weight and euthanized consecutively from 6 to 48 hours after inoculation. Mock-inoculated pigs served as controls. Body temperature was measured and blood samples were taken at regular intervals for bacteriology, haematology, clinical chemistry, and acute phase reactant determinations. Full necropsy was done and tissue samples were collected for bacteriology and histology. Apoptosis was measured in the spleen.

Results Onset of clinical disease (fever and lethargy) was seen at 7 to 8 hours after inoculation. Blood bacterial counts remained low throughout the study. SIRS characterized by fever, leukocytosis, increased levels of CRP, IL-6, IL-1β, TNFα, and decreased level of serum iron was detected after 12 hours. Both CRP and IL-6 levels peaked at 36 hours. Platelet numbers declined slightly and were lower than in the controls at 48 hours. Thromboelastography showed increased hypercoagulability over time. Levels of serum aspartate aminotransferase and bilirubin were elevated at 24 and 36 hours. Blood urea nitrogen levels had increased at 36 hours; however, no difference was seen in serum creatinine levels. Disseminated microabscesses were found in the lung at 6 hours, but had disappeared at 48 hours. In the bones, the presence of microabscesses progressed until 48 hours. Other histopathological signs related to inoculation were limited to a renal microabscess at 12 hours, splenic microabscesses at 24 hours and centrilobular hepatic necrosis with thrombosis in one animal at 48 hours. In the liver and kidneys, various degrees of fibrinous exudation were found. The number of apoptotic cells in the splenic white pulp was increased at 48 hours.

Conclusions All infected pigs developed sepsis with metastatic abscesses and at 48 hours severe sepsis was present with signs of dysfunction of the liver and the coagulation system. The splenic apoptotic response indicates reduced function and immunosuppression.

P39

Cyclin-dependent kinase inhibitor r-roscovitine reduces lipoteichoic acid lung inflammation and improves the resolution of antibiotic-treated *Streptococcus pneumoniae* pneumonia

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Introduction *Streptoccocus pneumoniae* pneumonia remains associated with high morbidity and mortality. Antibiotic treatment frequently is insufficient in limiting lung damage due to inflammation. Therefore, additional treatment strategies are needed. The drug r-roscovitine, a cyclin-dependent kinase (CDK) inhibitor, was demonstrated to reduce inflammation in several models of inflammation.

Objective We studied the potential of r-roscovitine to modulate host defense during sterile inflammation and bacterial infection of the lung.

Methods Isolated neutrophils were treated with 20 μM r-roscovitine and CDK and caspase 3 activity were determined by western blot analysis. Sterile lung inflammation was induced by intranasal administration of 100 μg lipoteichoic acid (LTA), a prominent cell wall component of Gram-positive bacteria. Simultaneously 70 mg/kg r-roscovitine or vehicle was injected intraperitoneally. Twenty-four hours later bronchoalveolar lavage (BAL) was performed and differential cell counts were determined. Bacterial pneumonia was induced by inoculation of 5 x 10^4 CFU *S. pneumoniae*. r-roscovitine (70 mg/kg) or vehicle was administered 24 hours later in combination with antibiotic therapy (ceftriaxon; 20 mg/kg). Mice were sacrificed after 48 hours. In a second experiment, mice were infected and treated at 24 and 72 hours and sacrificed 96 hours post infection.

Results r-roscovitine treatment significantly reduced phosphorylated CDK substrate and increased cleaved caspase 3 levels in isolated neutrophils. During LTA-induced lung inflammation, r-roscovitine treatment significantly reduced the amount of PMNs in the BAL fluid and cytokines in lung homogenates. After 48 hours of bacterial pneumonia, r-roscovitine-treated animals displayed enhanced pulmonary bacterial outgrowth. Cytokine production and lung damage scores were higher in the r-roscovitine-treated group as compared with vehicle. Interestingly, when studying the animals at 96 hours post infection, r-roscovitine treatment resulted in lower bacterial outgrowth and chemokine levels in the lung.

Conclusions With this study, we reproduced earlier findings that r-roscovitine treatment reduces CDK activity and induces apoptosis in neutrophils; we demonstrated that r-roscovitine diminishes inflammatory responses in sterile inflammation; and we found that r-roscovitine treatment in bacterial pneumonia is detrimental early in infection but beneficial at later time points. We believe that the negative effect of r-roscovitine reflects the importance of neutrophil antibacterial defense early in infection. Yet, during resolution of infection, apoptosis of neutrophils induced by r-roscovitine could present a way of damage control as opposed to unwanted side effects of neutrophil function.

P40

Honey as an immunomodulator during sepsis in animal model

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Introduction Malaysian honey (Gelam) has antibacterial activity and it also has a high antioxidant capacity and free radical scavenger activities. Honey extracts showed potent activity against TNFα in L929 cell and NO in RAW 264.7 macrophage as well as inhibitory effects on the prostaglandin E_2 and nitric oxide (NO) in inflammatory tissues of rat. Sepsis is mediated in part by bacterial endotoxin, which stimulates macrophages/monocytes to sequentially release early (for example, TNF, IL-1) cytokines and inducible enzymes such as inducible nitric oxide (iNOS) synthase and heme oxygenase 1 (HO-1) and late such as high-mobility group box 1 (HMGB1).

Objective To investigate the role of honey as an immunomodulator in sepsis induced by LPS in rats.